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Do Selye's Mammalian "GAS" Concept and "Co-stress" Response Exist in Plants?

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The logic behind the hypothesis of the putative existence of a general adaptation syndrome (GAS) in plants is that any plant—be it seasonal, annual, or perennial—during its life cycle is exposed to wide seasonal or diurnal vicissitudes of environmental conditions. During noon hours it may be challenged with heat stress while at night with chilling or freezing stress—when young it may develop and thrive in low temperatures when being exposed to a measure of root anoxia due to excessive soil moisture, while later it experiences partial desiccation and heat shock, but nevertheless it develops normally. Some evergreen perennials undergo cyclic changes of extremely cold winters and hot summers; several desert species undergo cyclic and marked changes of soil salinity that in a given location, as in a wadi-bed, is inversely related to precipitation.

Over and above these, plants may be faced with "climatic aberrations" in the form of hot, dry winds in early spring or cold spells in summer. Pollutants impose acute or chronic stresses on plants. It thus appears that an inherent multiple stress resistance mechanism is developmentally advantageous and may be pleiotropically encoded by evolutionary selection.

Environmental stresses are geared to circadian rhythms in plants. This hypothesis states that in plants, most of the biochemical and physiological processes manifest rhythmicity characterized by time periods ranging from seconds to annual seasons.[1] Of these, the most significant fluctuations are the circadian/diurnal and the ultradian oscillations that govern daily changes in physiological processes. Based on data pertaining to salinity stress, activities of antioxidant substances, and enzymes (glutathione reductase, catalase, peroxidase, superoxide dismutase), and findings that environmental stress marked altered rhythmic responses to UV-β tolerance, K^+ uptake, stomatal movements, nutrition, and resistance to iron stress, it is contended that duration, and more often amplitude, of rhythmic parameters is influenced by extreme environmental factors.[2]

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Concomitantly, it is suggested that stress injury in plants, at least in part, is caused by the desynchronization of the concerted rhythmicity established and entrained by the extant predominating environmental factors. In these terms, the stressor brings about modification and desynchronization of oscillations of biochemical and physiological processes by acting either on the input (from receptor to oscillator) or on the output (from the oscillator to the oscillating component) pathways. This view is in agreement with the criteria of the Selyean theory of stress, including the GAS hypothesis.[3] Thus, *adaptation* means re-setting of diurnal rhythms of biochemical/physiological processes and of the restoration of their concerted action, ensuring maximal resistance of the organism under the new conditions. This extension of the stress concept contributes to the recent revival and acceleration of the development of the Selyean theory of stress.[4,5]

GAS IN PLANT SYSTEMS

In the original surmise that GAS may apply to plants,[4] detailed documentation was presented indicating that *all* of the following groups of factors—abscisic acid (ABA) and/or jasmonic acid (JA) derivatives, $NO^{\bullet}$ and cytochrome P_{450} enzymes, free radical scavengers and antioxidants, osmoregulation, heat-shock proteins (HSPs), ubiquitin and chaperone complex, and (in certain cases) ethylene—are significantly involved in coping mechanisms when plants are challenged by the following environmental stress categories: anoxia, drought, heat, chilling, flooding, salinity, desiccation, and freezing.

These common denominators of stress coping strategies, cutting across diametrically opposed conditions (e.g., heat and freezing, desiccation and flooding), strongly suggest a Selyean process in the plant kingdom. In addition to the categories highlighted here, other GAS components unquestionably exist. Some of these will be discussed in the following sections.

ABA—THE PLANT'S ALL-ROUND STRESS REMEDY?

The most conclusive evidence of a GAS manifestation in plants applies to ABA. Typical examples include the alleviation of all environmental stress categories mentioned above as well as the hypersensitive (HS) response in phytopathological stress. An elegant and detailed treatment of ABA's role in stress coping is given elsewhere.[6]

The mode of ABA action in each stress type may differ, for example, in water stress ABA causes stomatal closing, which in turn may be associated with biophysical changes in guard cell membranes,[7] whereas in freezing tolerance ABA may serve to activate cold-regulated (*cor*) genes for cryoprotectant peptides.[8] Moreover, cDNA gene constituents for endogenous ABA synthesis have been found that are activated upon exposure to a wide spectrum of stress conditions.[9] In addition to having a biological effect on plant tissue, ABA may exert biophysical kosmotropic effect, which stabilizes the quasi-crystalline form of cell water.[7]

However, care should be taken not to overemphasize the role of this hormone in stress coping since ABA also induces senescence, and the delicate equilibrium

between stress-coping and senescence-evoking effects may tend toward the latter, especially in phase III of the Selyean stress curve (FIG. 1).

JASMONATES AND GAS

Jasmonates—JA, methyl jasmonate (MJ), and other related derivatives—are produced from α-linolenic acid (18:3) released from membranes when plants experience wounding or certain types of stress and, by several mechanisms, slow down several metabolic processes. Because dormant, nongrowing tissues are generally more resistant to stress injury than succulent, rapidly growing tissues, this series of effects is probably best viewed as a complex of protective processes designed to minimize further injury to the plant. A dormancy-related stress-coping effect is winter or summer tuberization, which precedes onset of seasonal-imposed climatic conditions that preclude above-ground plant growth. A close derivative of JA, tuberonic acid, has been shown to induce tuberization in several

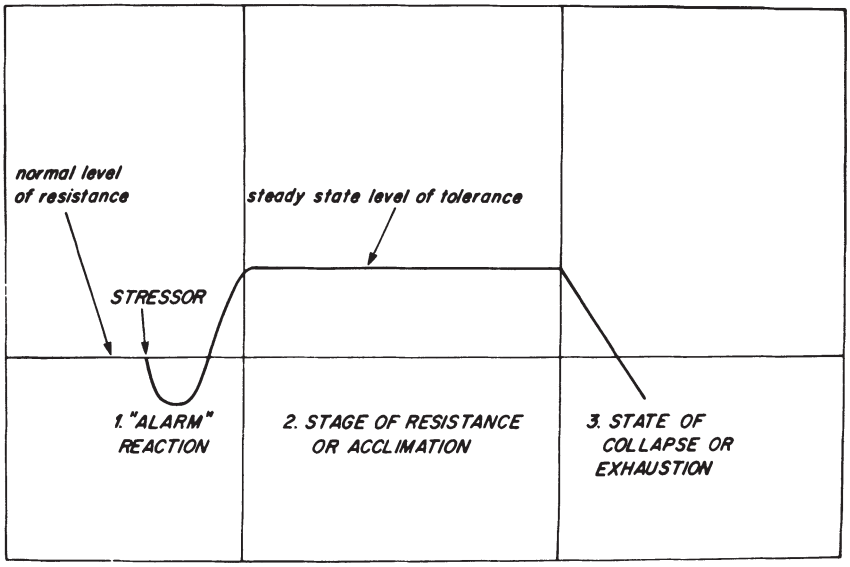

FIGURE 1. The three stages of the general adaptation syndrome (GAS). (Modified from Selye[3] to include plant systems.) ***1***—alarm reaction: this is the somatic response to the first exposure to a localized or general stressor and initially lowers basal state of resistance. If the "stressor" is sufficiently strong (e.g., extremes of temperature, salinity), death may result; **2**—stage of resistance or acclimation: if continued exposure to the stressor is compatible with adaptation, resistance mechanisms of either a "syntoxic" (coexistence with stress) or a "catatoxic" (stressor-removal) nature ensues. The cellular manifestations characteristic of the alarm reaction have virtually disappeared or are strictly held in abeyance, and resistance rises above normal; ***3***—state of collapse or exhaustion: following long-term exposure to the stressor, adaptation capability is eventually exhausted. Signs of the alarm reaction reappear, and the organ or the whole organism senesces and dies. Lichtenthaler[5] optimistically adds a fourth stage—that of "recovery" after exhaustion.

plant species including potatoes and dahlias. Jasmonates also play an overall role in disease resistance and are believed to act by regulating gene activity that induces novel proteins such as jasmonate-specific proteins (JSPs).[10]

OSMOREGULATION

Osmoregulation by synthesis of low molecular mass organic compounds in the cytoplasm of and accumulation of ions in the vacuole, follows the GAS hypothesis to a large extent: different stress signals such as frost, drought, and salinity induce an identical response, namely, synthesis of osmoregulators. Common to the mentioned stress signals is their effect by causing cellular dehydration. It should be emphasized that the stress signals—drought, salinity, and freezing—strongly differ in intensity.

A site of osmoregulant action is in association with the aqueous layers present on either side of membrane bilayers. Osmoregulants in these membrane-encasing aqueous layers, as well as in intramembrane "vicinal water," or in the "trapped" water layer present between each of the individual monolayers, may lend greater stability to the membrane and enable the withstanding of various types of stress categories.[11] The amount of hydration depends on lipid constituency, phase, and temperature and on hydrophilicity of membrane proteins. TABLE 1 indicates comparative hydrophilicities of membrane components.

Organic osmoregulators predominantly fall into four groups: (i) polyalcohols (polyols) such as glycerol, sorbitol, mannitol, and arabitol; (ii) nitrogen-containing compounds such as proline and betaine; (iii) saccharides including raffinose family oligosaccharides and fructans; (iv) sterols.

Osmoregulation is also expressed at the intact plant level. When plant roots are exposed to conditions that reduce water uptake, such as drought, salinity, frost, low root temperature, and hypoxia by water-logging, a signal in the form of ABA (or other compounds) is transferred to the shoot, resulting in stomatal closure and re-establishment of pressure potential by foliar synthesis of osmotic compounds. Stomatal closure by the ABA signal from the roots is caused by a reduced osmotic potential of the guard cells due to loss of K^+, Cl^-, and malate. Again, the GAS hypothesis is evident: tolerance to limiting water supply of the roots occurs, irrespective of the nature of its limitation. In a like manner, osmoticum and ABA interact to endow white spruce seedlings with drying and freezing tolerance.[12]

A special case of stress-associated carbohydrates is represented by the fructans. Fructan-accumulating plant species are distributed over dry and cold environments, and the level of fructans greatly increases under cold and dry conditions. Fructan accumulation confers drought tolerance in transgenic tobacco plants con-

TABLE 1. Degree of Hydration of Various Plant Membrane Lipid Constituents[11]

Membrane Lipid Component	Moles H_2O/Mole Lipid
Glucosylceramide	1
Sterolglycosides	1
Phosphatidylcholine (PC) (L β-gel phase)	8
Phosphatidylethanolamine	<15
PC (L α-liquid crystalline phase)	15
Monogalactosyldiacylglycerol	17
Digalactosyldiacylglycerol	34

taining microbial fructosyltransferase genes and high molecular weight microbial fructans.[13] On a molar basis high molecular weight fructan from microbial origin is nearly 30 times more effective in this respect than trehalose, sucrose, and glucose, indicating a specific role for fructans in stabilizing plant cell membranes under osmotic stress.

Membranes containing free sterols are involved in the sensing of osmotic shock of the salt tolerant alga *Dunaliella*, which responds to osmotic stress by synthesis of glycerol,[14] thus implying that plasma membrane sterols are clearly involved in osmoregulation of this halotolerant alga.

GAS, FREE RADICAL SCAVENGING, AND ANTI-OXIDATIVE MEASURES

That free radical scavenging, apparently without exception, is associated with every type of plant stress formerly listed, and this indicates a clear-cut general adaption function of this mode of stress coping, this including the $O_2^{\bullet}$-associated HR response.[15] SOD genes are differentially regulated and respond to a variety of stress conditons, such as paraquat application, drought,[16] and chilling.[17]

Catalase efficiently scavenges H_2O_2 to release water and dioxygen in peroxisomes and mitochondria. Catalase cDNAs have been cloned from several plants. The *CAT-2* gene of *Nicotiana* is induced by UV-B, ozone, and SO_2,[18] while stress factors such as heat and chilling may lead to catalase inactivation. Acclimation to chilling by pre-exposure treatments probably includes induction of catalase.[19] Recently, a role for H_2O_2 and catalase in the HR and SAR responses to plant pathogen infection has been proposed.

Notwithstanding the stated above, oxidative damage in plant stress may be a *consequence* and not a primary *cause* of stress. Thus, active oxygen deactivation may be a palliative measure taken by the plant to mitigate damage already caused.

STRESS ETHYLENE AND NITRIC OXIDE

As is well documented elsewhere, ethylene production accompanies all of the various plant stress categories. In aquatic species, however, ethylene promotes stem elongation by lowering endogenous ABA levels and hence increasing of sensitivity.[20] In senescing and water-stressed *Pisum sativum* and carnation tissue, it has recently been shown that low levels of the endogenously produced nitric oxide free radical ($NO^{\bullet}$) can inhibit ethylene emission.[21] The mechanism suggested is via the inhibition of the "ethylene-forming enzyme," ACC oxidase. Recent experimentation has extended this mechanism to salinity and short-term heat stress.

CHILLING RESISTANCE AND GAS

Chilling injury occurs in plants when exposed to suboptimal temperatures, the injurious suboptimal temperature depending on whether the plant is of tropical, subtropical, or temperate origin. Failure of the cellular membranes to maintain

compartmentalization of cellular contents either during or following low temperature leads to common symptoms of chilling injury such as pitting, water soaking, and increased ion leakage. A period of acclimation at a temperature close to, but above that which causes injury, sometimes suffices to prevent this damage. This cold acclimation involves the synthesis of a specific subset of proteins resulting from differential gene expression. In addition to acclimation, it was found that a similar degree of chilling resistance could be obtained at normal temperatures by water stress or ABA treatment.[22] Water stress can also increase freezing tolerance in winter rye and cabbage, as well as drought and freezing tolerance.

Chilling resistance can also be induced by high NaCl concentrations.[23] Chilling-sensitive plants such as tobacco and cucumber become more tolerant of low temperatures after being treated with NaCl. Low temperature tolerant plants such as spinach, wheat, and barley increase their freezing tolerance after exposure to increasing concentrations of NaCl in hydroponic culture over several days. There appears to be an overlap in the subset of genes induced by these stresses. Many, but not all, of the genes induced by acclimation are also regulated by water stress, salt stress, or ABA.[22]

"Temperature conditioning" of many postharvest commodities for a few days at temperatures near those that cause chilling injury allows adaptation and subsequent storage at low temperatures with little or no damage.[24] In contrast to acclimation, stress treatments such as heat, anoxia, and JA are being tried in postharvest situations to induce chilling resistance.[25–27] These treatments induce different subsets of genes, and yet all can increase chilling resistance in fruits when applied before low temperature storage. In the case of heat stress, there is a correlation between the persistence of heat-shock proteins (HSPs) in fruit tissue and chilling tolerance.[28] It may be inferred that the same is true in the other stress situations. Heat stress, by inducing chilling resistance, is moreover efficacious in many chilling-sensitive commodities including avocado, citrus, cucumber, mango, and zucchini.

Low oxygen stress, or anoxia, enhances chilling resistance in both avocado and mango. In addition to induction of anaerobic stress proteins, the anoxia treatment also enhances the level of antioxidants in avocado fruit during storage.[27] Because oxidative damage is involved in the development of chilling injury, high antioxidative capacity in tissue will prevent this damage. This may be one mechanism by which an anoxia stress will induce chilling resistance.

INTERACTION BETWEEN HEAT-SHOCK PROTEINS, UBIQUITIN, AND CHAPERONIN AND THE GAS RESPONSE

As in the case of other stress-coping factors, most, if not all, environmental stress-coping factors involve heat-shock proteins (Hsps). Especially important is the ubiquitin function, since in the final outcome all stresses cause dysfunction of proteins and since ubiquitin serves in their disposal, a presumptive overall GAS role, albeit secondary or tertiary, may well be assigned to Hsps.

In general, Hsps active in thermotolerance belong to the low-molecular-weight (LMW) Hsp class, and recently Hsp70 is arousing much interest. The mode of Hsp-mediated stress coping is pleiotropic and may involve mRNA protection, prevention of enzyme—especially photosynthesizing—denaturation and/or their stress-induced aggregation and post-stress ubiquitin and chaperonin-aided

repair.[29,30] More details of Hsp function in a context beyond the scope of the present review are given in Part I of this volume.

METAL POLLUTION

There is a large genetic variation in trace metal tolerance between plant species and between plant populations, the variation depending on the metal composition of the soil and rock substrate as well as environmental pollution stemming from industrial wastes.[31] Trace metals may enter plant cells via the same systems that carry essential metals, and populations may exhibit tolerance to a single metal species or to many.[6] Possibilities for trace metal tolerance are (i) binding to pectin residues and carboxyl groups in cell walls and (ii) complexing to organic acids in the cytosol followed by removal to the vacuole.[32] From the viewpoint of GAS detoxification of trace metals, metal binding peptides, phytochelatins and membrane-located pumping mechanisms appear to be the most important mechanisms.[31,33]

However, the role of phytochelatins in trace metal detoxification has recently been questioned. Although not contesting the presence and function of phytochelatins in non-stressed circumstances, it is claimed that coping with toxic levels of trace metals probably depends more on membrane-located H^+ATPase-associated pumping mechanisms that may remove excess metal cations from sensitive subcellular sites.[31] In conclusion, production of phytochelatins is a GAS reaction to trace metal exposure; however, production of phytochelatins is unsuitable as a sole parameter for screening for a particular trace metal tolerance, since it probably goes hand-in-hand with other stress-coping mechanisms.

A newly discovered mechanism found to be active in Al tolerance in transgenic tobacco and papaya is secretion by roots, of citric acid which binds Al in the soil, thus preventing it from entering and damaging the plant.[34] This appears to be a specific, and not a GAS, response.

CONCLUSIONS AND CAVEATS

This review mainly deals with physiological and biochemical aspects of GAS and co-stress; however, mention is made of some anatomical adaptations. These include foliar trichomes (hairs), which function efficiently in coping with varying stress situations: in water stress by reducing transpiration ("boundary layer effects"); high-light intensity and UV-B stress by filtering out harmful solar spectra; alleviation of heat stress by light interception and shading; prevention of pest infection by preclusion of insect probosci insertion into mesophyll tissue; and enhancement of freezing tolerance by reducing incidence of ice nucleation centers found in substomatal cavities. These and other anatomical adaptations relating *inter alia* to plant cuticulae, nature and chemical composition of the epidermis, degree of thylakoid thickness or curvature, anatomical preclusion of "acidosis" by microsiphoning off of excess CO_2,[35] and so forth, all of which are beyond the scope of the present review, may be as important in the GAS mechanism as the biochemical adaptions detailed above.

SUMMARY

Converging data indicate the possible existence of a general adaptation syndrome (GAS) in which different types of stress evoke identical coping mechanisms. In Selyean terms, this implies a "co-stress" response whereby one type of stress resistance may impart co-resistance to others. Common coping denominators may be physiological or morphological. The former include oxy-free radical scavenging, osmoregulation, ABA, jasmonates, chaperones, HSPs, and phytochelatins. Morphological GAS adaptations include leaf pubescence, movements and stance, and rooting characteristics. The feasibility, with certain reservations, of the GAS hypothesis is discussed here.

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